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ON THE HAEMATOPORPHYRIN OF SOLECURTUS STRIGILLATUS. By C. A. MAC MUNN, M.A., M.D. (Pl. XI.)

Solecurtus strigillatus is a mollusk which belongs to the family Solenidae. There are twenty-five species of *Solecurtus* known and the specimens referred to in this paper came from the South coast of France. Like the nearly allied "Razor-fish" *Solecurtus* buries itself in sand and mud, but whether the colour of *Solecurtus strigillatus* is "protective" or not I cannot say.

The specimens which I have examined had been preserved in spirit. On looking at such a specimen one is at once struck with the peculiar colour which is brown and the whole surface of the animal is more or less coloured except where it is attached to the shell. The foot is less deeply coloured than the siphons and that part of the body at their base. The surface of the foot is peculiarly mottled and on close examination the epithelial covering presents a very finely reticulated surface, the lines forming the mesh-work being more deeply coloured than the minute spaces they enclose. Where the foot is attached to the body the surface is only feebly tinged brown. The annulated siphons are more deeply coloured between the rings, and the portion of the body from which they arise is mottled a dark somewhat reddish brown.

On examination with the microscope the pigment is seen to be distributed—in the foot especially—in the manner shown in Pl. XI. fig. 1 from a photomicrograph, which shews that the bulk of the pigment occurs in the lines forming the mesh-work; these appear to mark the boundary between the epithelial cells or islands and this appearance recalls to mind the so-called endothelium cells stained with nitrate of silver.

On careful focussing it is seen that the spaces enclosed by the mesh-work are also themselves dotted with pigment and that the appearance of the double line forming the meshes is produced by

the coloured margins of adjacent epithelial cells which present a finely serrated appearance, the serrations being deeply pigmented; a very narrow transparent border occurs still more externally to these serrated margins, which appears to be the real outline of each epithelial cell or island; this is seen well under a $\frac{1}{4}$ objective. Tortuous channels free from pigment are enclosed between the borders of adjacent cells. Some of the cells are channelled by incomplete indentations each edge of which appears to be serrated, but the serrations being separated from the lumen of the channel by a fine transparent margin. In some places the epithelial surface is merely channelled, the edges of the channels as before having a serrated appearance, but the surface is not mapped out into islands owing to the channels being incomplete.

In the siphons the distribution of pigment is different, the edge of each ring being deeply pigmented so that the space enclosed between successive rings appears dark; the approximation of the rings not being complete leaves however a narrow bright channel between the rings as shown in fig. 2. The edges of the rings are wavy and under a quarter-inch objective are serrated, while the rings themselves are channelled more or less incompletely as shewn in figs. 2 and 3; in fig. 3 however being slightly out of focus in order to shew the granular appearance of the pigment between them. The margins of these channels are also serrated. The pigment in the spaces lying between these channels is seen under a high power to be deposited in well-defined elongated granules, some of which however recall to mind bone lacunae and under a $\frac{1}{16}$th objective magnifying about 1030 diameters this resemblance is more complete, as they are stellate, but the stars do not send out long rays. The darker granules under this magnification are also themselves seen to contain very fine brown granules and on the whole when examined by a sufficiently high magnifying power the granular character of the pigment is everywhere apparent.

If we compare these appearances with those presented by the dorsal streak of the earthworm which contains a similar pigment as I have already shewn[1], we find that in the latter case the pigment is not distributed in a mesh-work but without any definite arrangement, although here too the apparently amorphous masses of pigment are seen under a high power to be made up of granules.

The Spectroscopic Characters of the Pigment. Pl. XI. Fig. 4. The pigment is limited entirely to the surface of the animal and if a

[1] This *Journal*, Vol. VII. No. 3.

portion deeply enough coloured to give distinct bands be mounted in balsam after dehydrating in the usual manner, examined with the microspectroscope and compared with a similarly mounted specimen of Moseley's polyperythrin[1], e.g. from *Flabellum*, the bands are found to be identical both as regards position and relative shading. They are also identical with those in a similarly prepared specimen from the dorsal streak of *Lumbricus terrestris*. If the coloured portions be extracted with alcohol acidulated with sulphuric acid and the alcohol then filtered a purple red solution is obtained which gives the acid haematoporphyrin spectrum well marked, Pl. XI. Fig. 4 (sp. 2). The bands of which read as follows:

1st band λ 607·5 to λ 593,
2nd band λ 585 to λ 576 = shading,
3rd band λ 567·5 to λ 542,
4th band λ 523 to λ 506 = darker part.

By digesting coloured portions in rectified spirit and ammonia a faintly yellow solution was obtained shewing the four bands of alkaline haematoporphyrin; but a much better method is to dissolve the *isolated* pigment in rectified spirit and ammonia. For this purpose a solution of the pigment in rectified spirit and sulphuric acid is diluted with water and agitated in a separating funnel with chloroform when after some time the chloroform carries down the pigment forming a fine purple red solution, which seemed to possess a violet fluorescence. Thudichum noticed in the case of a chloroform solution, of what he calls "neutral fluorescent cruentine," a red fluorescence and he remarks[2]: "This is the first body which is known to fluoresce with homogeneous light, that is to say, the same kind of light or colours which it transmits." This chloroformic solution still shewed however the acid haematoporphyrin bands reading:

1st band λ 607·5 to λ 593,
2nd shading λ 585 to λ 577,
3rd band λ 567·5 to λ 543·5.

If now the residue left by evaporation of the chloroform be dissolved in rectified spirit with a little ammonia we get a spectrum which evidently denotes a mixture of acid and alkaline haematoporphyrin (sp. 3). The rose-red solution giving the following bands:

[1] *Quart. Journ. Micros. Soc.* Vol. xvii. 1887, pp. 1—23.
[2] Tenth Report, *Med. Off. Priv. Council*, 1867. Published 1868, p. 228.

1st a shading from λ 615 to λ 604,
2nd a darker part from λ 604 to λ 595,
3rd a shading from λ 583 to λ 567·5,
4th a band from λ 567·5 to λ 547,
5th a shading from λ 547 to λ 521·5.

But if more ammonia be added the spectrum changes into that of alkaline haematoporphyrin while the red colour of the solution is much less marked. (See sp. 4.) The bands of this solution read as follows:

1st band λ 636 to λ 622 (shading to λ 619),
2nd shading λ 607·5 to λ 601

(this shading was not seen in similar solutions of haematoporphyrin, possibly owing to greater dilution),

3rd band λ 591 to λ 564 (including shadings),
4th band λ 549 to λ 526,
5th band λ 515·5 to λ 488 (?).

If the above measurements be compared with those given for alkaline haematoporphyrin in my former paper in this *Journal*, Vol. VII. No. 3, no doubt can be felt that the pigments are identical.

If the chloroformic solution obtained by agitating a dilute acidulated-alcohol extract of the pigment in a separating funnel with chloroform be evaporated on the water bath, the residue obtained is not quite dry, and it is best to again redissolve it in spirit and again agitate with chloroform, on evaporating this at the temperature of the air we obtain an amorphous brown-violet residue, but this has still a somewhat greasy appearance and on dissolving it in absolute alcohol it is found to react acid owing to the clinging of the acid to the chloroform (which probably accounts for its solubility in the solvents mentioned below).

This residue appears to have a slightly greenish tint by daylight but purple-brown or brown-violet by gas-light and is partly soluble in ether, in carbon disulphide, in chloroform and benzol, insoluble in water.

If we place side by side the wave-length measurements of the bands of an acidulated alcohol extract of (1) haematoporphyrin obtained from sheep blood, (2) the present pigment, (3) the haematoporphyrin of *Uraster rubens*, (4) that from Slugs, and (5) that from *Lumbricus terrestris*, we can see at a glance the agreements and differences:—

Acid Haematoporphyrin[1].

	(1) *from Blood.*	(2) *Solecurtus.*	(3) *Uraster.*	(4) *Slugs.*	(5) *Lumbricus.*
1st band	605 to 591	607·5 to 593	607 to 591	600 to 591	603 to 591
2nd shading	583 to 567·5	585 to 576	583·5 to 576	582 to 572	583 to 575
3rd band	567·5 to 542	567·5 to 542	566 to 545·5	561·5 to 547	567·5 to 542

Of course a very slight difference in the degree of dilution would cause considerable differences in these measurements, and allowing for this the agreement is wonderfully close, so close as to place beyond doubt the identity of these pigments.

Hence then *Solecurtus strigillatus* owes all its brown colour to the presence of haematoporphyrin. And this may also be considered identical with Moseley's polyperythrin.

It would be interesting to know if this mollusk contains haemoglobin or histohaematins; but the specimens which I have examined were not suitable for determining this as they had been kept in spirit. Prof. Lankester[2] has found haemoglobin in the nearly allied *Solen legumen* in which it occurs in special corpuscles of its blood.

I thought I might meet with haematoporphyrin in *Solen siliqua* but obtained a negative result. For although the foot in parts is peculiarly marked somewhat like the foot of *Solecurtus* yet the pigment present is not haematoporphyrin, as it does not yield any to acidulated alcohol. In the same species I could not certainly determine the presence of histohaematins.

The presence of haematoporphyrin may now be considered as definitely decided in the following Invertebrates: *Ceratotrochus diadema, Flabellum variable, Flabellum* sp.?, *Fungia symmetrica, Stephanophyllia formosissima, Stephanophyllia* sp.?, an Actinia with a coriaceous test, *Discosoma* sp.?, and in *Cassiopeia*, in all of which as I said in a former paper Prof. Moseley found polyperythrin. I have found it in *Uraster rubens, Arion*

[1] The bands of alkaline haematoporphyrin vary according to concentration of solution much more than those of acid haematoporphyrin.

[2] *Proc. Roy. Soc.* Vol. xxi. (1872), p. 71, *et seq.*

ater, *Limax flavus* and other species, *Lumbricus terrestris* and lastly in *Solecurtus strigillatus*.

In *Rhizostoma Cuvieri* the blue colouring matter gives some bands which might be confused with those of polyperythrin but I find they are different, a result which agrees with that of Krukenberg who calls this pigment Cyaneïn and figures its spectrum[1].

The results arrived at in this and in my former paper in this *Journal*, Vol. VII. No. 3, confirm the conclusions of Sorby and Ray Lankester as to the occurrence of haemoglobin derivatives in various Invertebrates, if no other proof had been forthcoming. But the universal distribution of the histohaematins[2] and the fact that these yield some of the decomposition products of haemoglobin, as I have recently shewn in this *Journal*, Vol. VIII. No. 2, have fully explained the occasional appearance of a haemoglobin derivative in Invertebrates. This affords an answer to the following sentence in Krukenberg's *Grundzüge einer Vergleichenden Physiologie der Farbstoffe und der Farben*, namely: "Unbeirrt durch die Vorurtheile und die Sucht einzelner Untersucher, in jedem rothen, braunen oder dunkelgrünen Pigmente—befinde es sich an Infusorien gebunden in den Flüssen bei Guatemala (Rossignon), in Algen (Phipson) oder irgendwo bei Thieren (Ray Lankester, Sorby, Mac Munn)—ein verkapptes Hämoglobinderivat zu entdecken" and so on. (It is unnecessary to give the remainder of the passage.) To this I can now reply that Ray Lankester and Sorby were perfectly correct in their inferences and the occasional occurrence of such derivatives whether they are immature pigments or in process of disappearance can be easily explained. Their absence in certain species seems more difficult of explanation than their presence.

EXPLANATION OF PLATE.

Plate XI.

Figs. 1—3. From photomicrographs showing the distribution and morphology of the haematoporphyrin in the epidermis of *Solecurtus strigillatus*.

Fig. 1. From the foot, magnified 90 diameters.

Fig. 2. From the siphons also magnified 90 diameters.

Fig. 3. Part of the same magnified 280 diameters. The darker parts in all these figures represent the greatest accumulation of pigment. (For full explanation see paper.)

[1] *Vergleichend-physiol. Studien*, Zweite Reihe, dritte Abth. 1882, s. 68.

[2] *Philosophical Transactions*, Pt. I., 1886.

Fig. 4. *Spectra of Haematoporphyrin from Solecurtus strigillatus.*

1. Spectrum of a bit of epidermis at base of siphons of *Solecurtus strigillatus.*

2. Rectified spirit and sulphuric acid extract of the pigment, shewing bands of Acid haematoporphyrin.

3. The pigment was isolated by chloroform as described in the paper, the solution evaporated and the residue dissolved in rectified spirit to which a little ammonia was added; the spectrum is that of a mixture of acid and alkaline haematoporphyrin.

4. To the solution mentioned more ammonia was added, when the spectrum of 3 changed to this; that of alkaline haematoporphyrin. The very narrow and faint band before *D* is not constant in solutions of haematoporphyrin.

Fig 1 ×90.

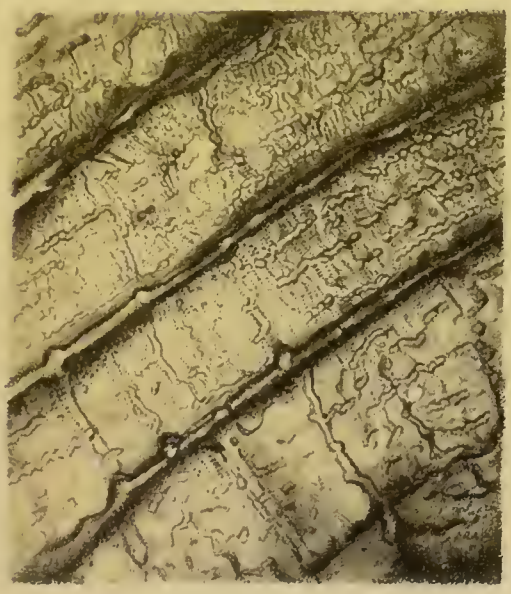

Fig. 2 ×90.

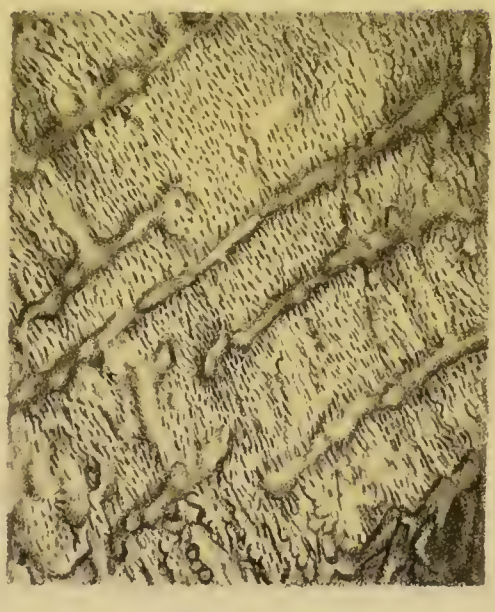

Fig. 3 ×280.

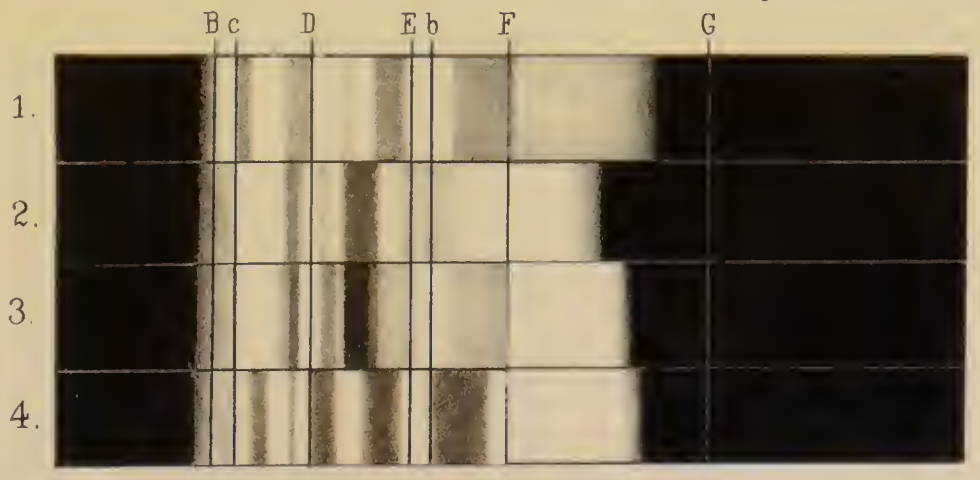

Fig. 4.

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